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Photosensory Proteins as a Tool in Synthetic Biology: Bridging Computational Biophysics and Systems Biology

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1 Introduction

The systems biology approach to understand basic questions related to health, food and the environment can be applied using different levels of molecular detail. Often it is claimed that systems-biological descriptions are based on ‘first principles’. Actually it will still take several decades before such an approach may become feasible (that is if ‘first principles’ is assumed to have its regular meaning in chemistry). Currently, at best, the most detailed elements of system descriptions are in terms of enzyme-kinetic characteristics (i.e. K_M and V_{max}). This approach, useful as it may be, is poorly suited for use in a synthetic biology approach. The latter would ideally integrate molecular dynamics modeling and molecular engineering in a systems description. Inclusion of this level of detail is important for applications in molecular medicine, e.g. when new enzyme inhibitors are to be designed.

When it comes to analyzing functional sub-states during enzymatic activity, photosensory proteins are the “star actors of modern times”¹. For important classes of photoreceptors both experimental measurements and molecular dynamics simulations of both the receptor state and the functional signaling state are available and mutually reinforcing. ‘Proof of principle’ for this approach has been obtained with e.g. Photoactive Yellow Protein^{2,3}. The predicted structure of the signaling state of this protein, calculated with molecular dynamics simulations, including the parallel tempering approach, turned out to correlate well with the results obtained with NMR measurements of the solution structure of this (sub) state. In ongoing work, transition-path sampling is used to analyze the key factors that govern the transitions between the relevant sub-states of this photoreceptor.

Light is a very convenient substrate, not only in photoreceptor studies, but also in experimental work on cellular biological systems. It is therefore surprising that not more systems biology modeling work has been done on biological systems containing photosensory proteins. As a result very little progress has been made in method development to incorporate light in a systems biology approach. Here we have developed a method based on the Lambert-Beer law. To test this method we used light-regulated gene expression via the BLUF-domain of the AppA protein from *Rhodobacter sphaeroides*. The AppA protein is involved in the blue-light repression of photosynthesis genes⁴⁻⁶. *In vitro* experimentation has shown the photocycle of AppA can be summarized in a simple photocycle scheme with only 2 main components^{7,8}, the receptor state R and the signaling state S (see Fig. 1a).

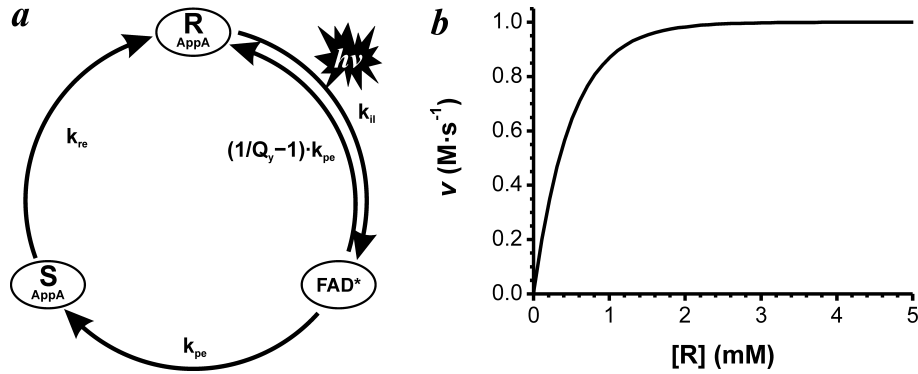


Figure 1. Panel a: Representation of the AppA photocycle model, with R the receptor state, FAD* the excited state upon light absorption, and S the signaling state. Panel b: Rate of excited state formation as function of the R state concentration. This plot was generated using equation 3, where $V = 1 \text{ cm}^3$; $l = 1 \text{ cm}$; $I_0 = 1 \text{ mmol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$; c is the concentration of R in M. Note that [R] is plotted in mM and that k_{il} ranges from $2 \cdot 10^3$ to $2 \cdot 10^2 \text{ s}^{-1}$ for [R] is 0.01 to 5 mM.

The signaling state is formed on a nanosecond timescale, whereas the recovery from S to R takes place on a timescale of minutes.

2 Methods

2.1 Kinetic Model of the AppA Photocycle

The model depicted in Fig. 1a was used to test the incorporation of light in the AppA model. Here k_{pe} is the photocycle entry rate or the rate with which the signaling state is formed from the excited state. Q_y is the photocycle quantum yield or the fraction of excited states that go on to form the signaling state. k_{re} is the recovery rate or the rate with which the receptor state is formed from the signaling state. k_{il} is the illumination rate constant. This rate constant is dependent on both the light intensity and the concentration of the sample. Derivation of this rate equation is shown below. For the other reactions first-order rates were assumed, in accordance with the results of the time-resolved spectroscopy experiments. Calculations with the differential equations that describe the model depicted in Fig. 1a, were performed in MatLab 7.1.

2.2 Derivation of Illumination Rate Constant (k_{il})

For the derivation of the illumination rate constant we assumed that for each photon that is absorbed, a receptor state protein is converted to the excited state. By subtracting the intensity of light used to illuminate the sample (I_0) by the intensity of light after it has passed through the sample (I) we can determine the amount of light that was absorbed. By choosing the unit of light intensity as $\text{mmol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ ($= 10^{-7} \text{ } \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) dividing it by the illuminated sample volume (V) and subsequently multiplying it with the area (a) of the sample that is illuminated, the velocity (v) is obtained with which the excited state of the

photoactive protein is formed. From this velocity an illumination rate constant (k_{il}) can be determined.

$$A = \log \frac{I_0}{I} = \varepsilon \cdot l \cdot c \quad (1)$$

$$(I_0 - I) = I_0 - \frac{I_0}{10^{\varepsilon \cdot l \cdot c}} = I_0 \cdot \left(1 - \frac{1}{10^{\varepsilon \cdot l \cdot c}}\right) \quad (2)$$

$$v = c \cdot k_{il} = \frac{a}{V} \cdot I_0 \cdot \left(1 - \frac{1}{10^{\varepsilon \cdot l \cdot c}}\right) \quad (3)$$

$$k_{il} = \frac{I_0 \cdot \left(1 - \frac{1}{10^{\varepsilon \cdot l \cdot c}}\right)}{l \cdot c} \quad (4)$$

Equations 1-4 depict the different steps of the derivation, where A is absorption; I_0 and I are light intensities before and after absorption by sample ($\text{mmol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$); c is the sample concentration (M); ε is the Molar extinction coefficient of the sample ($\text{M}^{-1} \cdot \text{cm}^{-1}$); l is the path length of the light through the sample (cm); a is the area of the sample that is illuminated; V is the volume of sample illuminated ($V = a \cdot l$; cm^3); v is the velocity with the excited state of the sample is formed ($\text{M} \cdot \text{s}^{-1}$); k_{il} is the illumination rate constant (s^{-1}).

3 Results and Discussion

The k_{il} is linearly dependant on light intensity, however the dependence on the sample concentration is a little more complex as is illustrated in Fig. 1b, where rate of excited state formation v ($c \cdot k_{il}$) is plotted as a function of sample concentration. Note that v varies in the concentration range of 0 to 2 mM which is equivalent to a sample OD of 0 to 17 of the flavin absorption peak at 446 nm. This falls not only within the biologically relevant range but also within the range in which *in vitro* measurements are generally performed.

The model depicted in Fig. 1a was tested on data where the N-terminal 5-125 AppA fragment was placed in a spectrophotometer and bleaching of the sample by the spectrophotometers probe light was followed in time. The model was able to accurately describe the data. The values for k_{re} and I_0 were fitted to the data and, within the error of the measurements, resulted in the same values as those that were measured (Data not shown).

Having established that the model can accurately describe the photocycle of AppA, it was used to simulate the effect of k_{re} on the light sensitivity of the protein. In Fig. 2 a simulated light titration of the N-terminal 5-125 AppA fragment and the W104A mutant of the same protein fragment is depicted. The major difference here is the value for k_{re} which is almost 200 times faster for the W104A mutant⁹. The graph clearly shows that in the case of the W104A mutant much more light is needed in order to obtain similar amounts of the signaling state. This has implications for studies on the biological function of such a mutant, as one might mistakenly come to the conclusion that the mutant has lost its function (e.g. by analyzing its function only at $100 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), whereas in reality the used light intensity was insufficient to activate the mutant.

The essential part of this photocycle model has been incorporated in an overall description of light-mediated regulation of gene expression via AppA (see Fig. 3). In ongoing experiments we are analyzing the light intensity dependence of *puf* expression in intact

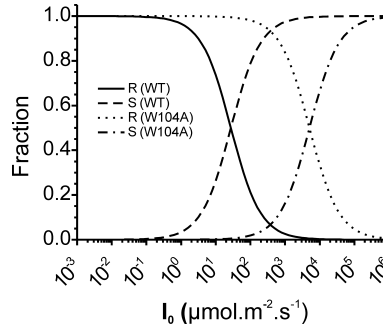


Figure 2. Simulation of the light sensitivity of AppA. Fractions of the R and S state of AppA at equilibrium are plotted as function of the used light intensity. This plot was generated with the photocycle model depicted in Fig. 1a, with $a = 1 \text{ cm}^2$; $l = 1 \text{ cm}$; $Q_y = 0.24$; $k_{pe} = 10^9 \text{ s}^{-1}$; $\epsilon_{400-700\text{nm}} = 884 \text{ M}^{-1} \cdot \text{cm}^{-1}$; $[\text{AppA}] = 60 \text{ } \mu\text{M}$. I_0 was varied from 10^{-3} to $10^6 \text{ } \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. To simulate the N-terminal 5-125 AppA Wild type fragment a k_{re} of 0.0013 s^{-1} was used. For the W104A mutant a k_{re} of 0.24 s^{-1} was used.

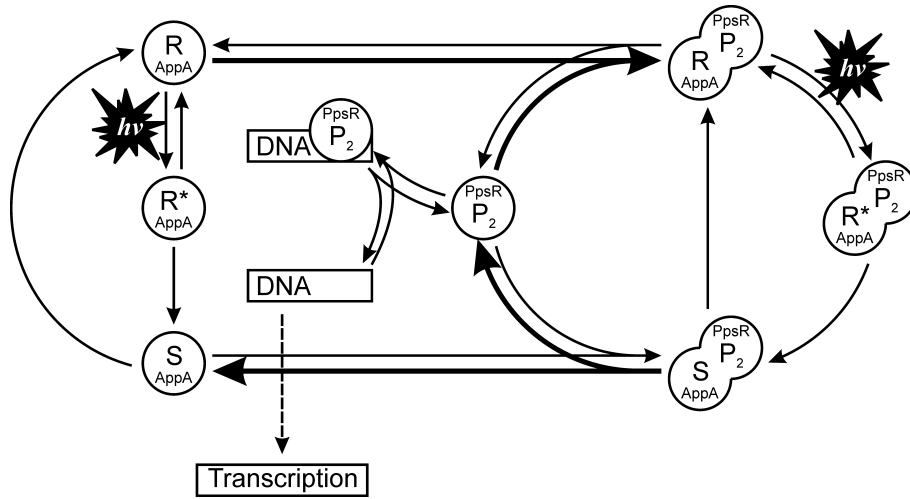


Figure 3. Model for light-mediated regulation of gene expression via AppA.

cells of *Rhodobacter sphaeroides*. In this approach we will compare the wild type protein with several of its site-directed mutants (including the W104F mutant).

We are also setting up a similar model for the YtvA mediated blue-light induced activation of the σ^B -dependent stress response of *Bacillus subtilis*¹⁰. This system makes use of very large ($> 10 \text{ MDa}$) signaling complexes (so-called ‘stressosomes’), which generates very interesting questions regarding sensitivity, cooperativity, etc., in this signal transfer pathway. Furthermore, the light-sensitivity of YtvA provides an ideal handle to analyze the linkage of the signal transduction network that activates σ^B , with other (late growth) signal transduction networks in this organism.

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